Acute Effects of Oral Caffeine Intake on Human Global-Flash mfERG Responses: A Placebo-Controlled, Double-Masked, Balanced Crossover Study

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Purpose. To determine the acute effect of caffeine intake on the retinal responses as measured with a global-flash multifocal electroretinogram (gfmERG) protocol at different contrast levels.

Methods. Twenty-four young adults (age = 23.3 ± 2.4 years) participated in this placebo-controlled, double-masked, balanced crossover study. On two different days, participants orally ingested caffeine (300 mg) or placebo, and retinal responses were recorded 90 minutes later using a gfmERG at three contrast levels (95%, 50%, and 29%). The amplitude response density and peak time of the direct and induced components (direct component [DC] and induced component [IC], respectively) were extracted for five different eccentricities (1.3°, 5.0°, 9.6°, 15.2°, and 21.9°). Axial length, spherical equivalent refraction, habitual caffeine intake, and body weight were considered as continuous covariates.

Results. Increased IC amplitude response density was found after caffeine ingestion in comparison to placebo ($P = 0.021$, $\eta_p^2 = 0.23$), specifically for the 95% and 50% stimulus contrasts ($P = 0.024$ and 0.018, respectively). This effect of caffeine on IC amplitude response density was independent of the retinal eccentricity ($P = 0.556$). Caffeine had no effect on DC amplitude response density or DC and IC peak times.

Conclusions. Our results show that oral caffeine intake increases the inner electro-retinal activity in young adults when viewing stimuli of high- (95%) to medium-contrast (50%). Given the increasing evidence that the inner retinal function is involved in the emmetropization process, these results may suggest that caffeine or its derivatives could potentially play a role in the mechanisms involved in eye growth.

Keywords: myopia, emmetropization, adenosine receptors, inner retina, gfmERG

Myopia is widely recognized as a public health concern and a risk factor for multiple ocular diseases.[1] Myopic refractive errors are typically a consequence of the excessive axial growth of the eye during childhood. This excessive axial elongation is a result of the failure of the cascade of signals that regulate ocular growth starting at the retina.[2] Vision-dependent mechanisms trigger signals that modulate the biochemistry and tissue biomechanics of different eye structures (i.e., retinal pigment epithelium, choroid, and sclera), leading to alterations in the ocular growth.[3] Due to the significant socioeconomic and health consequences of myopia, there is a high interest in the scientific community to find more effective therapies to reduce the eye´s axial elongation.[4] A number of optical and pharmacological treatments are currently available, but these cannot fully stop the progression of myopia and do not work in all patients.[5] There is an urgent need to increase the limited efficacy of myopia control treatments in order to address the emerging epidemic of myopia.

Caffeine, a nonselective adenosine receptor antagonist, and its derivatives 7-methylxanthine (7-MX) have gained popularity as potential therapeutic interventions for myopia control.[6–10] Indeed, the oral administration of 7-MX has been demonstrated to reduce the axial myopic changes produced by either form deprivation or hyperopic defocus in a variety of mammalian animal models such as rabbits,[11] guinea pigs,[12] and monkeys.[13] Nevertheless, no effects were found for 7-MX on preventing induced myopia in tree shrews or chickens,[14,15] and a pilot study conducted in 2008 reported that the effects of systemic 7-MX were limited to children with moderate baseline axial growth rate (i.e., lower than 0.19 mm per six months) when treated for 24 months.[6]

A recent retrospective human study by Trier and colleagues[7] in Denmark assessed the rate of myopia progression in 711 children and showed that the association between myopia progression and 7-MX was dose-dependent, namely higher doses of 7- MX seemed to be more effective for myopia control. Smith et al.[8] also noted that the

effect of topically instilled caffeine in retarding axial elongation in infant rhesus monkeys was due to changes in axial length, not the optical components of the eye. They hypothesized that caffeine modulates the vision-dependent retinal cascade involved in the normal emmetropization process. Adenosine receptors are localized in the neural retina among other ocular structures, and they have been shown to have physiological effects that are relevant to the emmetropization cascade (i.e., the release of neurotransmitters involved in regulating ocular growth).[13,16] Caffeine is an adenosine receptor antagonist, and thus it is likely that this substance may partially influence the mechanisms involved in the emmetropization cascade.[17]

The inner electroretinal activity has been suggested as a valid measure to determine the retinal mechanisms linked to myopia progression because the inner retinal function is more susceptible to myopia.[18–23] The development of the global-flash multifocal electroretinogram (gfmERG) allows the assessment of inner retinal responses at different eccentricities.[24] Previous studies have found a functional loss at the inner retinal layers in myopic eyes,[19,22,23] as well as a response to optical defocus,[25] with these effects being dependent on eccentricity, contrast levels, and age. The suitability of using the gfmERG to determine the retinal mechanisms associated with myopia progression has fostered the investigation of different myopia control strategies, namely atropine and dual-focus contact lenses, on the human electroretinal response.[26–28] These studies observed a heightened inner retinal response with myopia control strategies, giving valuable information on the possible mechanisms of action in inhibiting ocular growth.

Given the accumulated scientific evidence on the potential value of caffeine and its derivatives for myopia control in humans[6–10] and the validity of the gfmERG response to objectively assess the retinal mechanisms associated with the emmetropization cascade,[18–23] the main objective of this placebo-controlled, double-masked, balanced crossover study was to examine the acute effects of orally ingesting 300mg of caffeine in retinal responses as measured by the gfmERG at three contrast levels and five retinal eccentricities. We hypothesized that acute caffeine ingestion would lead to greater inner retinal activity in comparison to placebo intake, as it has been shown for other myopia control strategies such as atropine and dual-focus contact lenses.[26–28] Also it is plausible to expect that these effects would be dependent on the stimulus contrasts and retinal eccentricity, as it has been shown in previous studies assessing the association between myopia and retinal function.[19,22,23,25]

Methods

Participants

The sample size for this study was calculated using an approximate value of gfmERG amplitude response density (from now on referred to as response density for brevity) that accounted for the direct and induced components (DC and IC, respectively) and the three contrast levels used in this study (0.30 ± 0.15 nV/deg^2). An effect size of 0.25 would allow us to detect differences lower than 15% (0.04 nV/deg^2), which were the differences obtained by Khanal et al.[28] when using atropine. Using a-priori sample size calculation, power of 0.80, and α of 0.05, the projected required sample size for this within-subjects design was 22 subjects. Twenty-four young adults (mean age $\pm$ SD 24.6 ± 2.4 years, age range 21–30 years, 17 females) were recruited from the New England College of Optometry (NECO) population to participate in this study. Participants were recruited through email invitation messages to the College's community. All participants met the following inclusion criteria: (i) no history or presence of ocular surgery or disease based on a thorough ocular history, slit-lamp and fundus examination, (ii) have a spherical equivalent between +0.75 D and −6.00 D and less than 3.00 D of astigmatism in each eye, as measured with the WAM-5500 open-field autorefractor (Grand Seiko Co. Ltd., Hiroshima, Japan), (iii) best corrected LogMAR visual acuity +0.10 (20/25 Snellen equivalent) or better in each eye, (iv) no history of adverse reactions to caffeine or diagnosis of epilepsy, (v) not being pregnant or nursing, and (vi) no allergies or sensitivity to tropicamide eye drops. The experimental protocol followed the guidelines of the Declaration of Helsinki and was approved by the NECO institutional review board. The purpose and details of the study were explained to each subject, and all questions were answered. After that, the subjects were asked to read and sign the consent form.

Experimental Design

We used a double-masked, placebo-controlled, within-subjects design to assess the acute effects of caffeine consumption on the retinal responses as measured with a gfmERG protocol at different contrast levels and retinal eccentricities. The within-participants factors were the caffeine consumption (placebo and caffeine), stimulus contrast (95%, 50%, and 29%), and stimulus eccentricity (Ring 1: 0°–1.3°, Ring 2: 1.3°–5.0°, Ring 3: 5.0°–9.6°, Ring 4: 9.6°–15.2°, and Ring 5: 15.2°–21.9°). The dependent variables were the response density and peak time of DC and IC. We also measured the participant's subjective level of activation with a visual analog scale.

Procedure

Participants were asked to attend the laboratory in two experimental sessions on two different days. The sessions were scheduled at the same time of day ($\pm$1 hour) to avoid circadian fluctuations. The experimental tests performed in both sessions were identical, with the only difference that either a caffeine (300 mg) or a placebo capsule were ingested at the beginning of the experimental session. The order of both experimental conditions (caffeine and placebo) and the three stimulus contrasts used in each session (95%, 50%, 29%) were randomized. The caffeine and placebo capsules had the same color and shape, and they were tasteless (i.e., encapsulated using hard gelatin capsules) and prepared/coded by a third person to accomplish the double-masked procedure. Regulatory bodies recommend that daily intake of caffeine should remain below 400 mg per day to avoid safety concerns for healthy adults,[29,30] and thus we considered it appropriate to use a caffeine content of 300 mg for this study. A serving of Americano or Espresso has an approximate caffeine content of 140–150 mg.[31,32] The chosen caffeine content (300 mg) represents two servings of these coffees, which are doses frequently consumed by adults.[33,34] In the first session, after participants read, understood, and signed the consent form, they were weighed, and completed a demographic and caffeine habits questionnaire. After that, they underwent an optometric examination in order to verify that the inclusion criteria were fulfilled. The examination included a slit lamp and ophthal-

moscopy examination to detect possible ocular pathology and a measure of intraocular pressure using a rebound tonometer (Icare IC200; TiolatOy, Inc., Helsinki, Finland). In addition, objective refraction was conducted using the WAM-5500 open-field autorefractor and subjective refraction with endpoint criteria of maximum plus sphere and minimum minus cylinder power, maintaining the best visual acuity. Charts using the Bailey-Lovie design were used to measure visual acuity and refraction. Lastly, axial length and other biometric components were measured using a Lenstar LS900 optical biometer (Haag-Streit AG, Koeniz, Switzerland) following the manufacturer's recommendations.

From this point, both sessions were identical. Before caffeine or placebo ingestion and to check that participants performed both experimental sessions under similar levels of attentiveness, participants were asked to complete the Stanford Sleepiness Scale questionnaire. This survey assesses perceived levels of sleepiness/alertness, and survey contains seven statements ranging from 1 "Feeling active, vital, alert, or wide awake" to 7 "No longer fighting sleep, sleep onset soon, having dream-like thoughts."[35] Participants were also asked to complete a visual analog scale to report their perceived level of activation at baseline and after 90 minutes of capsule ingestion (before starting the gfmERG recording). This numerical scale ranged from 1 "absolutely not activated" to 10 "extremely activated."

gfmERG Recording

All measures were performed in the right eye, and the left eye was occluded using an eye patch. After 70 minutes from the capsule (caffeine or placebo) ingestion, one drop of 0.5% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) was instilled into the tested eye. Given the short duration of our recordings (~20 minutes) a single drop of 0.5% tropicamide is sufficient to ensure appropriate dilation during the testing time.[36] Following standards from the International Society for Clinical Electrophysiology of Vision for multifocal electroretinography,[37] the ERGs were recorded with a corneal DTL (Dawson, Trick, and Litzkow) electrode placed along the lower eyelid margin of the right eye (Diagnosys LLC, Lowell, MA, USA), and after cleaning the skin with an alcohol pad and an abrasive paste, two skin electrodes served as reference (ipsilateral cantus of the tested eye, Ambu neuroline 700; Ambu, Ballerup, Denmark) and ground (subject's forehead, 3M Red Dot; 3M Corp, St. Paul, MN, USA). If required, electrodes were readjusted and repositioned to achieve an impedance of <5 kΩ. The recordings commenced after 20 minutes of tropicamide installation, after the examiner confirmed that the pupil of the tested eye was dilated to at least a 7-mm diameter. Pupil diameters were measured using a half-moon pupil gauge to reflect standard clinical practice[38,39] and through the Veris system (EDI Inc, Redwood City, CA, USA) (average pupil diameter of 7.75 ± 0.44 mm, ranging from 7.1 to 8.8 mm). Therefore the electrical activity of the retina was measured after 90 minutes of caffeine/placebo intake. This time was chosen based on the findings of Kamimori and colleagues,[40] who found that peak plasma caffeine levels occur between 84 to 120 minutes after caffeine ingestion.

The electroretinograms were recorded using the Visual Evoked Response Imaging System (Veris) approved by the Food and Drug Administration. The system uses the FMS III EDI stimulator (Electro-Diagnostic Imaging, Milpitas, CA, USA), with an LCD micro display viewed through an optical system. The FMS III stimulator has an integrated refractor that provides correction of spherical refractive errors from −8.00 to +6.00 D, whereas its optical system compensates for stimulus minification or magnification to ensure constant stimulus size irrespective of the refractive error of the observer. This is important for maintaining consistent stimulus eccentricities across all subjects.[37] Participants were asked to use the focusing knob on the stimulator to achieve a sharp image of the stimulus before starting the recording of a segment. Although the system does not correct for astigmatism, previous studies have shown that changing the magnitude and orientation of astigmatic blur has no effect on the response density or latency of the gfmERG response.[41] The astigmatic errors of the participants included in this study were smaller than those in previous studies (average standard ± deviation = −0.69 ± 0.46 D).

The screen subtended 44° × 37° of visual angle (H×W) at a viewing distance (through the optical system) of 40 cm. The stimulus pattern subtended ~44° of visual angle on the horizontal meridian and ~36° on the vertical meridian and consisted of 61 hexagons that were scaled to compensate for the reduction in retinal cell density with eccentricity (stretch factor 12.18). The stimulus was flickering in a pseudorandom binary sequence (m-sequence of 12), while the subject fixated on a red cross presented at the center. Three different stimulus contrasts were tested (95%, 50%, and 29%), all with a mean luminance of 75 cd/m^2. The inclusion of three different contrasts was based on the differences observed for gfmERG responses as a function of contrast stimulation in subjects with myopia.[22,23] The background and global flash luminance were 75 cd/m^2 and 164 cd/m^2, respectively. The stimulus sequence started with a multifocal stimulus frame, followed by a dark frame, a global flash, and a second dark frame in each cycle; the video frame rate was 75 Hz (Fig. 1). Participants used the focusing knob on the stimulator/camera of the Veris system to achieve a sharp image of the stimulus. Each contrast level of the ERG protocol lasted approximately 4 minutes, which was completed in 16 segments to minimize discomfort (each segment lasted approximately 15 seconds). Those segments contaminated by blinks or other artifacts were discarded and re-recorded. The signals were amplified using a Physiodata Amplifier system (15A54; Grass Technologies, Astro-Med, Inc., West Warwick, RI, USA). The bandpass filter was set at 10 to 300 Hz and the gain was ×100,000.

gfmERG Analysis

The gfmERG trace arrays were pooled into five concentric rings and averaged for analysis using the system software (VERIS; EDI, Inc.). Aiming to facilitate the comparison of our results with previous studies using multifocal procedures, the eccentricities chosen were similar to investigations assessing the association between myopia and gfmERG responses.[21,23,27] The first and second distinct peaks were defined as DC and IC, respectively. The DC response density was measured from the first distinct trough to the following peak, whereas the IC response density was measured from the second distinct peak to the subsequent trough. The DC peak time was measured from the onset of the multifocal flash frame to the first distinct peak. The IC peak time was measured from the presentation of the global flash frame (i.e., 26.6 ms) to the second distinct peak (Fig. 2).

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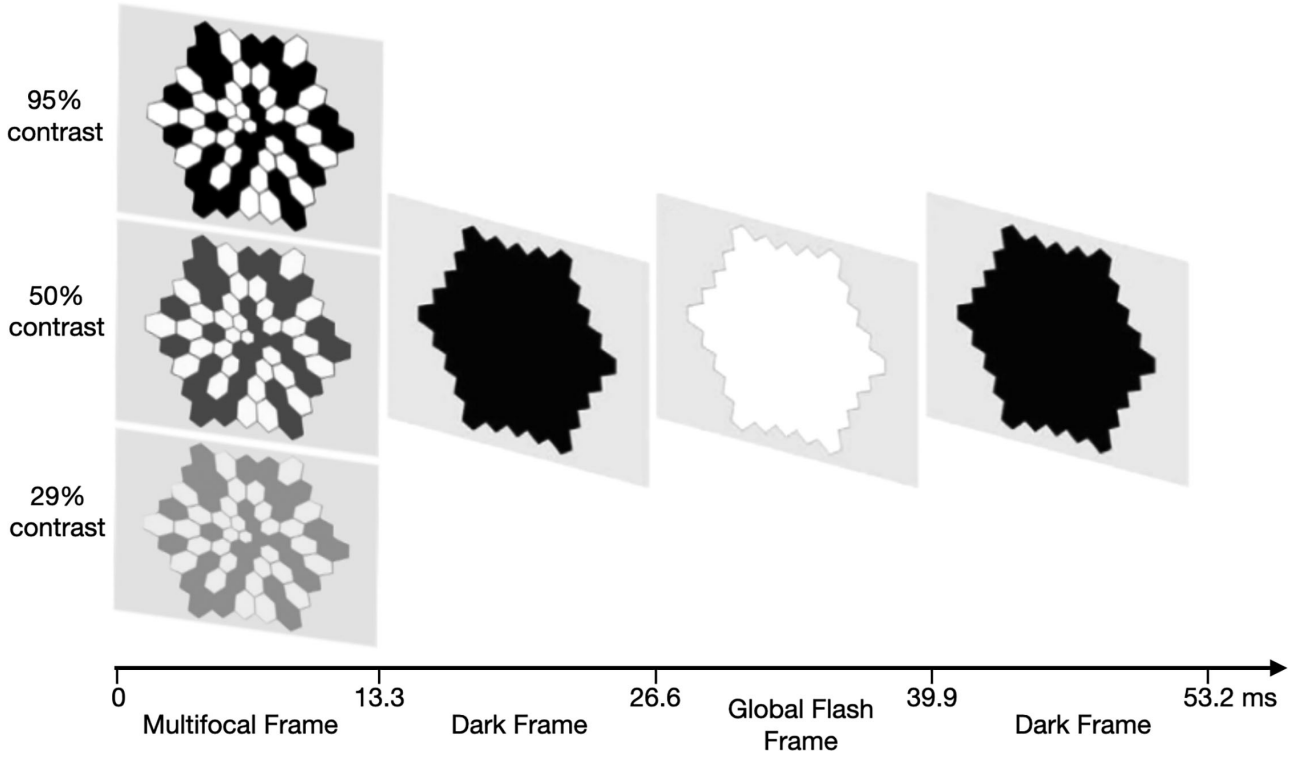

Figure 1. A schematic diagram showing the global flash multifocal electroretinogram paradigm at the three contrast levels used in this study. The paradigm was composed of four video frames: starting with a frame of multifocal stimulus (61-scaled hexagonal array), followed by a dark frame, a global flash frame, and a second dark frame. The monitor refresh rate was 75 Hz, and the frame interval was 13.3 ms.

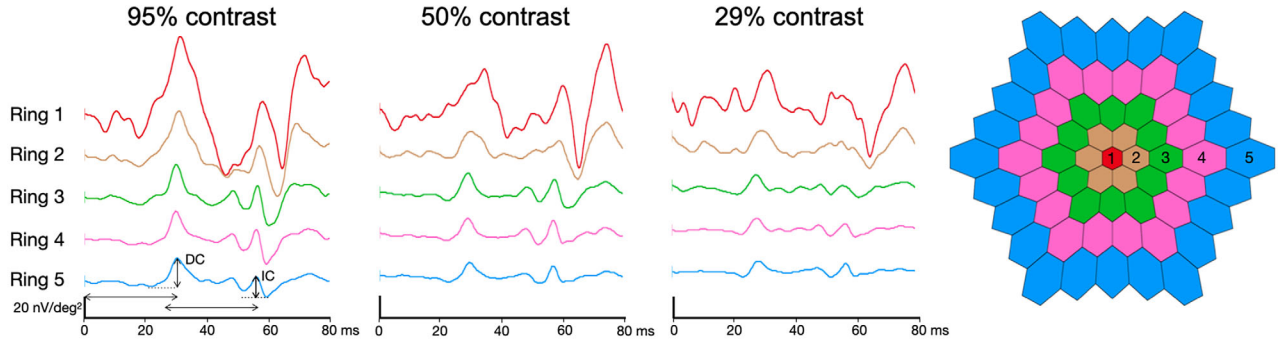

Figure 2. An example of the gfmERGs to three different contrast levels from one subject. The illustration on the right-hand side shows the 61 scaled hexagons used in this study. The different colors represent the five different ring groups (Ring 1: 0°–1.3°, Ring 2: 1.3°–5.0°, Ring 3: 5.0°–9.6°, Ring 4: 9.6°–15.2°, and Ring 5: 15.2°–21.9°) that were used for analysis.

Statistical Analyses

Shapiro–Wilk and Levene's tests were performed to assess the normality of the data and the equality of variance, respectively ($P > 0.05$). A *t*-test for paired samples (caffeine, placebo), considering the score obtained in the Stanford Sleepiness Scale, was performed to assess possible differences in the perceived levels of alertness/sleepiness before the commencement of both experimental sessions. To evaluate whether caffeine intake influenced the perceived levels of activation, a 2 (caffeine intake: caffeine, placebo) × 2 (point of measure: before, after 90 minutes) ANOVA, with the two factors manipulated within participants, was carried out.

Four separate within-participant analyses of covariance were carried out for the response density and peak time of the gfmERG DC and IC. For these analyses, the within-participant factors were contrast (95%, 50%, and 29%), caffeine intake (caffeine, placebo), and eccentricity (Ring 1, Ring 2, Ring 3, Ring 4, and Ring 5). The axial length, refractive error (i.e., spherical equivalent obtained in subjective refraction), habitual caffeine intake, and body weight were continuous covariates. For all analyses, violations of sphericity were managed by adjusting the degrees of freedom

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according to the Huynh-Feldt method, as implemented in the JASP statistical software (Version 16.4). An α of 0.05 was adopted to determine statistical significance of main effects, and the Holm-Bonferroni correction was adopted for multiple comparisons. Standardized effect sizes were reported as partial η^2 (η_p^2) and Cohen's d (d) for F and T tests, respectively.

Results

We confirmed that all participants attended the session under similar levels of alertness/sleepiness, as analysis of the Stanford Sleepiness Scale showed no statistically significant differences between the caffeine and placebo conditions (t = 1.23, p = 0.23, d = 0.25; caffeine = 2.67 ± 0.87, placebo = 2.96 ± 1.00). For the perceived levels of alertness, there were statistically significant differences for the main effects of caffeine intake ($F = 12.34$, $P = 0.002$, $\eta_p^2 = 0.35$) and point of measure ($F = 18.08$, $P < 0.001$, $\eta_p^2 = 0.44$), as well as for the interaction caffeine intake · point of measure ($F = 13.21$, $P = 0.001$, $\eta_p^2 = 0.37$). Specifically, we found greater levels of perceived alertness after 90 minutes of caffeine ingestion (mean change of 1.29 ± 1.30 and −0.21 ± 1.06 for caffeine and placebo, respectively).

Descriptive values for the gfmERG variables (i.e., response density and peak time of the DC and IC) considered in this study are shown in Table 1. Statistical parameters of the main and interaction effects for the four gfmERG variables assessed in this study are summarized in Table 2 (see also supplementary material).

Caffeine Intake

The main effect of caffeine intake revealed statistically significant differences for the IC response density, with higher values for the caffeine condition in comparison to the placebo condition ($F = 6.3$, $P = 0.021$, $\eta_p^2 = 0.23$). The interaction of caffeine intake × contrast was also statistically significant ($F = 4.6$, $P = 0.015$, $\eta_p^2 = 0.18$), with the increase in IC response density after caffeine intake in comparison to placebo being statistically significant for the 95% ($F = 5.8$, $P = 0.024$, $\eta_p^2 = 0.21$) and 50% ($F = 6.5$, $P = 0.018$, $\eta_p^2 = 0.22$) stimulus contrasts but not for the low-contrast (29%) condition ($F = 0.1$, $P = 0.846$) (Fig. 3). DC response density, DC peak time, and IC peak time did not show statistical significance for the main effect of caffeine or its interactions (Table 2 and Figs. 3, 4).

Contrast

There were statistically significant main effects of contrast on DC response density, DC peak time, and IC response density (all $P < 0.001$), but not for IC peak time ($P = 0.693$) (Table 2). The results showed higher values of DC response density with greater contrast levels, obtaining a higher DC response density for the 95% contrast in comparison to the 50% and 29% contrast conditions (corrected $P < 0.001$ in both cases, ds = 0.89 and 1.88, respectively), as well as for 50% contrast when compared with the 29% contrast condition (corrected $P < 0.001$, $d = 0.78$). Longer values of DC peak time were obtained for the 95% contrast in comparison to the 50% (corrected $P = 0.003$, $d = 0.74$) and 29% (corrected $P < 0.001$, $d = 1.49$) contrasts, as well as for the comparison

Table 1. Descriptive Values (Mean ± SD) of the Multifocal Electroretinogram Responses of the Global Flash Paradigm Obtained at the Different Eccentricities and Contrasts in the Caffeine and Placebo Conditions

	95% Contrast		50% Contrast		29% Contrast	
	Caffeine	Placebo	Caffeine	Placebo	Caffeine	Placebo
DC response density (nV/deg^2)						
Ring 1 (0°–1.3°)	47.34 ± 26.15	48.15 ± 23.56	34.24 ± 8.97	36.41 ± 13.64	28.18 ± 7.69	30.40 ± 8.19
Ring 2 (1.3°–5.0°)	27.23 ± 8.70	28.11 ± 10.87	26.56 ± 5.76	24.61 ± 8.24	23.71 ± 8.67	21.82 ± 8.80
Ring 3 (5.0°–9.6°)	21.39 ± 8.42	21.50 ± 8.84	18.95 ± 8.47	17.81 ± 8.30	13.24 ± 7.59	12.20 ± 7.85
Ring 4 (9.6°–15.2°)	19.22 ± 5.86	19.61 ± 7.32	16.02 ± 5.34	15.25 ± 5.89	11.67 ± 4.54	11.38 ± 5.56
Ring 5 (15.2°–21.9°)	16.80 ± 4.65	17.20 ± 6.30	14.38 ± 4.41	13.09 ± 4.49	10.60 ± 3.12	9.71 ± 4.25
IC response density (nV/deg^2)						
Ring 1 (0°–1.3°)	62.91 ± 31.47	55.93 ± 25.28	59.04 ± 28.32	52.29 ± 24.97	47.38 ± 22.41	46.82 ± 16.52
Ring 2 (1.3°–5.0°)	30.31 ± 11.99	27.56 ± 12.59	27.44 ± 11.70	24.93 ± 7.78	19.16 ± 7.09	19.95 ± 6.37
Ring 3 (5.0°–9.6°)	26.96 ± 11.40	24.15 ± 9.78	21.78 ± 6.53	19.95 ± 5.80	15.43 ± 6.39	16.68 ± 6.10
Ring 4 (9.6°–15.2°)	19.02 ± 9.13	14.22 ± 7.95	14.63 ± 5.10	11.96 ± 5.16	9.73 ± 6.85	10.87 ± 3.30
Ring 5 (15.2°–21.9°)	10.30 ± 5.92	5.38 ± 3.09	9.46 ± 3.91	5.13 ± 2.84	6.96 ± 2.74	6.10 ± 2.76
Peak time DC (ms)						
Ring 1 (0°–1.3°)	31.22 ± 1.80	31.77 ± 1.91	30.63 ± 1.27	31.06 ± 2.31	30.04 ± 2.97	30.94 ± 3.17
Ring 2 (1.3°–5.0°)	30.43 ± 1.54	30.85 ± 1.58	30.21 ± 1.11	29.88 ± 2.20	29.44 ± 2.00	29.73 ± 2.33
Ring 3 (5.0°–9.6°)	30.31 ± 1.22	30.64 ± 1.55	29.46 ± 1.19	29.39 ± 2.08	28.73 ± 1.32	28.73 ± 1.23
Ring 4 (9.6°–15.2°)	30.52 ± 1.31	30.87 ± 1.57	29.65 ± 1.30	29.67 ± 2.17	28.88 ± 1.35	28.73 ± 1.36
Ring 5 (15.2°–21.9°)	30.93 ± 1.27	30.96 ± 1.76	30.31 ± 1.44	30.15 ± 2.11	29.35 ± 1.53	29.48 ± 1.58
Peak time IC (ms)						
Ring 1 (0°–1.3°)	33.44 ± 1.49	33.37 ± 2.13	33.20 ± 2.62	33.35 ± 1.59	32.30 ± 2.18	32.62 ± 1.94
Ring 2 (1.3°–5.0°)	31.16 ± 0.95	31.26 ± 1.98	31.68 ± 1.97	31.70 ± 1.69	30.95 ± 2.50	30.68 ± 2.27
Ring 3 (5.0°–9.6°)	29.97 ± 1.06	30.00 ± 0.99	30.23 ± 0.98	29.80 ± 2.04	30.01 ± 1.36	30.25 ± 1.08
Ring 4 (9.6°–15.2°)	30.07 ± 1.83	29.54 ± 1.35	29.97 ± 1.05	29.72 ± 1.77	29.71 ± 1.21	30.04 ± 0.92
Ring 5 (15.2°–21.9°)	30.21 ± 1.88	29.68 ± 1.84	30.17 ± 1.43	29.79 ± 1.84	30.39 ± 1.20	30.25 ± 1.19

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Table 2. Statistical Values (F, P value and η_p^2) of the Main and Interaction Effects for the Four gfmERG Variables Assessed in This Study

	DC Response Density		IC Response Density		Peak Time DC		Peak Time IC	
	F	P (η_p^2)	F	P (η_p^2)	F	P (η_p^2)	F	P (η_p^2)
Main effects								
Caffeine intake	0.1	0.881 (0.01)	6.3	**0.021 (0.23)**	1.2	0.280 (0.06)	0.5	0.509 (0.02)
Contrast	30.9	**<0.001 (0.60)**	12.2	**<0.001 (0.37)**	23.1	**<0.001 (0.54)**	0.4	0.693 (0.02)
Eccentricity	164.1	**<0.001 (0.89)**	132.2	**<0.001 (0.86)**	17.4	**<0.001 (0.47)**	72.7	**<0.001 (0.79)**
Interaction effects								
Caf × Cont	0.3	0.763 (0.01)	4.6	**0.015 (0.18)**	0.2	0.800 (0.01)	1.4	0.259 (0.07)
Con × Eccen	6.8	**<0.001 (0.25)**	2.1	**0.038 (0.09)**	2.0	0.056 (0.09)	1.5	0.152 (0.07)
Caf × Eccen	0.5	0.746 (0.03)	0.7	0.556 (0.03)	1.3	0.607 (0.03)	0.3	0.883 (0.02)
Caf × Cont × Eccen	0.4	0.893 (0.02)	0.2	0.981 (0.01)	0.7	0.690 (0.03)	0.8	0.587 (0.04)

Caf, caffeine intake; Cont, contrast; Eccen, Eccentricity.
Bold text denotes statistically significant effects ($P < 0.05$).

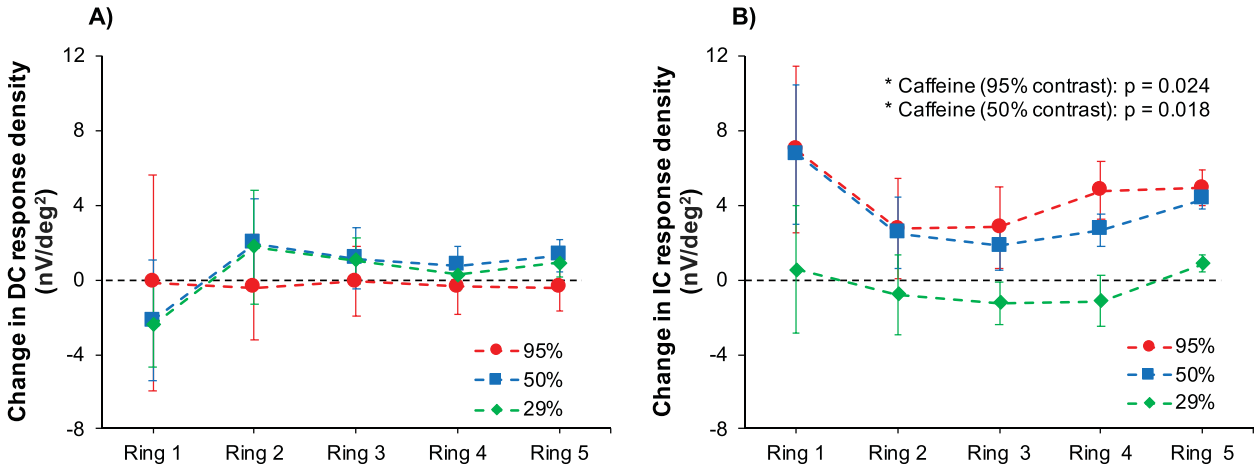

Figure 3. Changes in direct component (**A**) and induced component (**B**) amplitude response densities as a function of caffeine intake for the 95%, 50% and 29% contrast levels. The five retinal eccentricities considered are depicted in the X-axis (Ring 1: 0°–1.3°, Ring 2: 1.3°–5.0°, Ring 3: 5.0°–9.6°, Ring 4: 9.6°–15.2°, and Ring 5: 15.2°–21.9°). Change is calculated as the caffeine minus placebo value. P values for the statistically significant effects of caffeine intake at the corresponding contrast levels (95% and 50% for IC response density) are depicted. *Error bars* represent SEM.

50% contrast versus 29% contrast (corrected $P = 0.003$, $d = 0.75$). Greater IC response densities were obtained in the 95% in comparison to the 29% contrast condition (corrected $P = 0.00$, $d = 1.06$), as well as for the 50% in comparison to the 29% contrast condition (corrected $P = 0.006$, $d = 0.67$).

Eccentricity

The main effect of eccentricity yielded statistical significance for the four gfmERG variables (see Table 2). For DC response density, higher values were found at eccentricities closer to the fovea. Specifically, higher DC response density was found for Ring 1 in comparison to Rings 2, 3, 4, and 5 (corrected $P < 0.001$ in all cases, $ds = 2.41$, 3.92, 4.34, and 4.72, respectively), for Ring 2 in comparison to Rings 3, 4, and 5 (corrected $P < 0.001$ in all cases, $ds = 1,51$, 1.93, and 2.30, respectively), and for Ring 3 in comparison to Ring 5 (corrected $P = 0.001$, $d = 0.79$). The comparisons between Rings 3 and 4, as well as between Rings 4 and 5, did not reach statistical significance (corrected $P = 0.103$ in both cases). A longer DC peak time was found for Ring 1 in comparison to Ring 2 (corrected $P < 0.001$, $d = 1.01$), Ring 2 (corrected $P < 0.001$, $d = 1.17$), Ring 3 (corrected $P < 0.001$, $d = 1.66$), Ring 4 (corrected $P < 0.001$, $d = 1.44$), and Ring 5 (corrected $P = 0.002$, $d = 0.82$), as well as for Ring 2 when compared to Ring 3 (corrected $P = 0.020$, $d = 0.65$). Also, longer values of DC peak time were found for Ring 5 when compared with Ring 3 (corrected $P = 0.002$, $d = 0.84$) and Ring 4 (corrected $P = 0.024$, $d = 0.62$).

Regarding IC response density, higher values were obtained for Ring 1 in comparison to Rings 2, 3, 4, and 5 (corrected $P < 0.001$ in all cases, and $ds = 2.82$, 3.19, 3.90, and 4.49, respectively). In addition, Ring 2 exhibited a greater IC response density when compared to Rings 4 and 5 (corrected $P < 0.001$ in both cases, and $ds = 1.08$ and 1.67, respectively), as well as for Ring 3 in comparison to Rings 4 and 5 (corrected $P = 0.004$ and < 0.001, respectively, and $ds = 0.71$ and 1.30, respectively) and Ring 4 in comparison to Ring 5 (corrected $P = 0.015$, $d = 0.59$). Lastly, longer IC peak times were obtained for Ring 1 in comparison to Ring 2 (corrected $P < 0.001$, $d = 1.74$), Ring 3 (corrected $P < 0.001$, $d = 2.97$), Ring 4 (corrected $P < 0.001$, $d = 3.18$), and Ring 5 (corrected $P < 0.001$, $d = 3.02$), as well as for Ring 2 in comparison to Ring 3 (corrected $P < 0.001$, $d = 1.23$), Ring

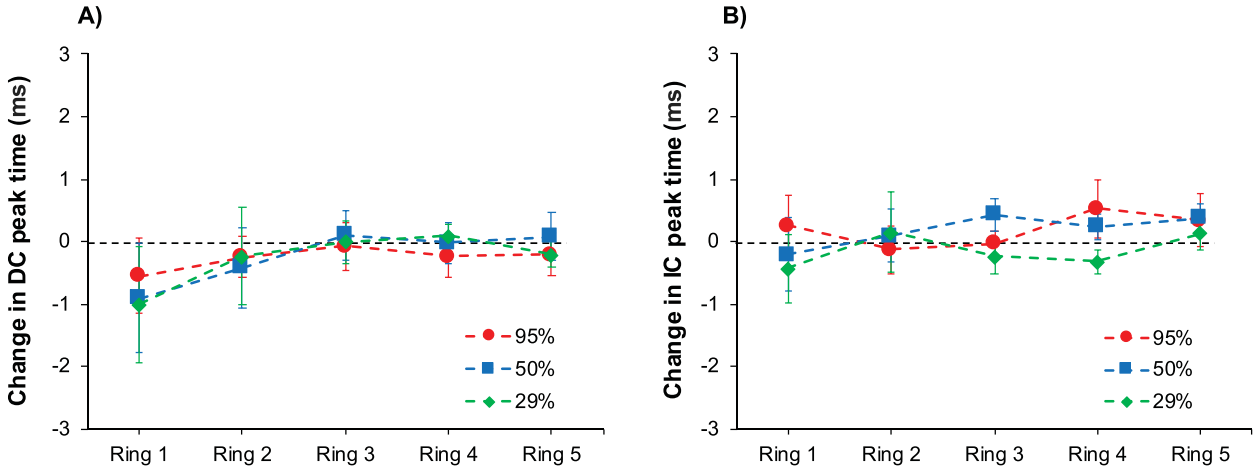

Figure 4. Changes in direct component (**A**) and induced component (**B**) peak times as a function of caffeine intake for the 95%, 50% and 29% contrast levels. The four retinal eccentricities considered are depicted in the X-axis (Ring 1: 0°–1.3°, Ring 2: 1.3°–5.0°, Ring 3: 5.0°–9.6°, Ring 4: 9.6°–15.2°, and Ring 5: 15.2°–21.9°). Change is calculated as the caffeine minus placebo value. *Error bars* represent SEM.

4 (corrected $P < 0.001$, $d = 1.44$), and Ring 5 (corrected $P < 0.001$, $d = 1.28$).

Covariate Analysis

No effects were found for the covariates refractive error, axial length, habitual caffeine intake, and body weight for DC response density ($P > 0.248$), DC peak time ($P > 0.073$), IC response density ($P > 0.864$), or IC peak time ($P > 0.269$).

Discussion

This placebo-controlled, double-masked, balanced crossover study was designed to examine the acute effects of caffeine on the outer and inner retinal function. These results show that ingesting 300 mg of caffeine affected inner retinal responses as measured with the gfmERG, when using stimuli of high- (95%) to medium-contrast (50%). In line with recent investigations assessing the short-term effects of two different myopia control strategies (i.e., atropine and dual-focus lenses) on the retinal responses,[26–28] our results reveal that caffeine intake modulates the inner retinal function, which may link caffeine with the retinal mechanisms associated with emmetropization. On the other hand, the differences found in the perceived levels of activation corroborate previous findings on subjective responsiveness to acute caffeine intake, namely, ingesting caffeine increases feelings of arousal.[42,43] Therefore the observed effects could also simply be due to the systemic stimulatory caffeine effect.

Differences in functioning and anatomic configuration of the central and peripheral areas of the retina are well known.[44–47] Walker et al.[48] observed that blur sensitivity, which is essential in the emmetropization process, is decreased in the near-peripheral visual field (i.e., 12° eccentricity) of myopic eyes. Additionally, studies with myopic subjects have demonstrated a reduced inner electroretinal activity at paracentral regions, with these effects being mostly observed at retinal eccentricities ranging between 5° and 20°, approximately.[22,23,25] Taken together, these results indicate that the near peripheral retina seems to play a more involved role in the emmetropization process than central areas of the retina. Our results show that the increase in the inner electro-retinal activity after orally ingesting 300 mg of caffeine do not differ between central and peripheral areas of the retina. A number of questions remain, including the effect of caffeine across different ages and refractive error groups. We recommend that future studies consider long-term interventions, the inclusion of different caffeine doses and other forms of administration (e.g., eye drops).

In the current study, the average percentage of the increase in the neural activity of the inner retina caused by caffeine ingestion was of 14.9% and 13.7% for the high- and medium-contrast conditions after 90 minutes of ingesting 300 mg of caffeine, respectively. However, the lack of effect of the low contrast stimulus on retinal responses might further highlight the role of inhibitory and contrast gain mechanisms in these processes.[49] The synthesis and release of dopamine in the retina is mainly attributed to amacrine cells,[50] which are necessary for regulating the emmetropization process.[51] Indeed, dopamine and its metabolites are known to be lower in myopic in comparison to non-myopic eyes and experimentally-induced myopia have demonstrated to cause a reduction in retinal dopamine concentration.[52,53] Interestingly, an endogenous increase in dopamine levels have shown to prevent form-deprivation myopia in mice[54] or the heightened release of dopamine caused by atropine instillation has been suggested as the mechanism responsible for controlling myopia progression with atropine.[55] Along the same line, the beneficial effect of spending time outdoors on myopia onset and progression seems to be mediated by the stimulatory effect of light on retinal dopamine production and release.[56] In relation to the physiological mechanism associated with the effects of caffeine on inner electro-retinal activity, and its potential utility for myopia control, it is well known that caffeine attenuates the negative effects of adenosine on dopamine receptors (i.e., stimulating dopaminergic activity).[57] Also, evidence from animal studies reveals that systemic administration of caffeine increases extracellular levels of dopamine and glutamate in the shell of nucleus accumbens[58] and alters the levels of adenosine receptors in the retina.[59] Therefore the modu-

lation of dopaminergic levels via caffeine intake, because it has been suggested for other myopia control strategies such as increasing the time spent outdoors[56] and atropine,[60] could be considered as a promising alternative for myopia control.

With regard to the plausible mechanisms underlying the caffeine-induced effects on the electrical activity of the retina obtained in this study, it is worth mentioning that previous studies have found a caffeine-induced vasoconstrictive effect on the retinochoroidal vasculature and alteration in the retinal morphology in the short-term.[61–63] For example, Dogan and colleagues[63] recently reported that caffeinated coffee, when compared to decaffeinated coffee, appears to transiently reduce parafoveal vessel density, capillary flow area, and subfoveal thickness in healthy young adults. In addition, studies conducted with different clinical populations (e.g., retinitis pigmentosa, diabetic retinopathy or glaucoma) have obtained a significant association between the electrical activity of the retina and the retinochoroidal vasculature and morphology, which evidences a link between morphological and functional changes in the retina.[64–67] Therefore it is plausible that the observed caffeine-induced effects on the inner retinal function may be partially explained by alterations in the retinochoroidal vasculature and morphology. Even though it is established that choroidal thickness is correlated with axial length and refractive error,[68–70] our covariate analysis indicates that caffeine effects on the inner retinal function occur irrespective of axial length and refractive error. Future studies should investigate this possibility.

We found that the ingestion of 300 mg of caffeine compared to placebo causes a significant increase of IC response density, as measured with a gfmERG protocol and when using stimulus of high (95%) to medium contrast (50%), in young adults, which may be of relevance due to association between the neural activity of the inner retina and myopia onset and progression.[18–23] However, there are some aspects that may be considered as a limitation of our findings, and they must be listed. Orally administered caffeine rapidly crosses the blood-brain barrier, inducing a mild stimulant effect.[71] This increase in behavioral arousal can be prevented by other adenosine antagonists such are 7-MX or topically instilled caffeine.[8,9] Future studies are required to determine compounds and doses that allow to sufficiently stimulate the dopaminergic activity of the retina without causing undesirable effects, mainly in children who are the targeted population for myopia control. Caffeine anhydrous has been demonstrated to alter the ocular physiology, for example, causing vasoconstriction in retinal arterioles and venules of the eye,[61] rising intraocular pressure[43] or altering the morphology and biomechanics of the cornea[72,73] and the dynamics of ocular accommodation.[74] Thus the possible effects of 7-MX or topically instilled caffeine on the ocular morphology and vasculature and its potential association with changes in the retinal function should be tested in future studies. Also, the physiological response to caffeine is subject to tolerance,[75] and it requires consideration when assessing the potential utility of caffeine and its derivatives for myopia control. Additionally, we need to acknowledge that we cannot discern whether the increase in the inner-retinal activity is due to a local effect of caffeine on the retina or due to its global/systemic stimulatory effect. Our experimental sample was limited to healthy young adults with refractive errors ranging from 0.50 to −6.00 D (mean spherical equivalent ± standard deviation: −1.67 ± 1.97 D) and included 14 individuals with myopia (spherical equivalent ≤−0.75 D). The applicability of the results of this study to other populations (those with hyperopia, children) requires further investigation. Lastly, two recent studies have found the non-genotoxicity and non-mutagenicity of repeated doses of 7-MX, suggesting that it can be considered as clinically safe.[76,77]

In conclusion, our results imply that 300 mg of orally ingested caffeine increases the inner electroretinal activity when viewing stimuli of high (95%) to medium contrast (50%) in young adults, with these effects being independent of retinal eccentricity. Future research is needed to explore the clinical applications of caffeine and other adenosine receptor antagonists for modulating the inner retinal function.

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